UPFLOW ANOXIC SLUDGE BLANKET REACTOR WITH GRANULAR PHENOLYTIC DENITRIFIERS FOR PHENOL REMOVAL

A Thesis Submitted

in Partial Fulfilment of the Requirements

for the Degree of

MASTER OF TECHNOLOGY

by

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to the

DEPARTMENT OF CHEMICAL ENGINEERING

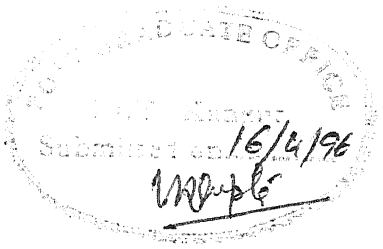
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It is certified that the work contained in the thesis **"Upflow Anoxic Sludge Blanket Reactor with Phenolytic Denitrifiers for Phenol Removal"**, by *Anupam Kansal*, has been carried out under our supervision and that this work has not been submitted elsewhere for a degree.

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ACKNOWLEDGEMENT

I am greatly indebted to my guides, Prof. A.B.L. Agarwal and Prof. C. Venkobachar for their valuable guidance.

I express my deep sense of gratitude to Dr. A.B.L. Agarwal for the motivation and personal attention through out my thesis work. His compassion guidance enabled me to complete the course.

I express my deep sense of gratitude to Dr. C. Venkobachar. His guidance during my course work and thesis helped me in understanding the biological processes in a better way. I am grateful to him for the valuable time spared for me and help in all respects.

Thesis work was impossible without the involvement of Dr. (Mrs.) Leela Iyengar. The granular biomass provided by her made it possible to conduct the present study. I express my sincere gratitude to her.

The suggestion given by Mr. M.G. Grasius in fabrication and running of the reactor were very much valuable. I am highly thankful to him for the help extended to me.

The suggestions and help provided by Ligy Philip, Pavitra Sandilliya, Rathi, Verma made my task simpler.

The gas chromatographic analysis was not possible without the help of Mr. K.K. Pant, research scholar in Dr. Kunzru's lab.

I remember with love, the pleasant and memorable company of Nitin A. Gawande. He has always been ready to come to my help.

The help and company of Ramakrishna, Yadav and Ansari ji in environmental engg. lab made my experimental study a cheerful and memorable experience.

Finally, I thank all other friends of mine who made my stay in I.I.T., Kanpur, comfortable.

- Anupam

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ABSTRACT

Hazardous phenolic effluents emanated by many industries like petrochemicals, steel industry, petroleum refineries, resins & chemicals manufacturing are reported to be amenable for biological treatment. A laboratory scale upflow anoxic sludge blanket (UASB) reactor of 1.35 litres capacity was fabricated and seeded with granular sludge which was developed using enriched heterogeneous phenolytic denitrifiers isolated from soil. The performance of UASB reactors was investigated at various influent phenol concentrations ranging from 600-1100 mg/L & residence times ranging from 2 to 24 h.

Results indicated that granular phenolytic sludge could easily be developed and maintained for a long time, in a medium containing phenol as sole organic carbon source and nitrate as electron acceptor. The phenolytic biomass could sustain its metabolic activity upto a phenol concentration of 1000 mg/L and sludge loading rate of 0.26 g COD/g VSS-d. Influent concentration beyond 1000 mg/L is toxic to the phenol degrading organisms. For the influent phenol concentration of 750 mg/L, the reactor performed satisfactorily upto 3 h hydraulic retention time (HRT) but failed at 2.5 h as observed by the increased COD, phenol, NO_3-N, NO_2-N & TSS Concentration in the effluent. For a phenol concentration of 600 mg/L, the reactor performance was satisfactory upto 3 h HRT and failed at HRT of 2 h. The stoichiometry for organic carbon and NO_3-N removal was calculated to be equal to 1.15 : 1.

1. INTRODUCTION

Water which is so essential for the sustainence of life can become scourge for the very existence of life when it is contaminated with domestic and industrial wastewatwers. The industrial activities often result in the release of chemicals into the surroundings that have severe public health and environmental consequences. Many industries such as petrochemicals, steel, resin and chemicals emanate hazardous phenolic effluents.

The most commonly used methodologies for the removal of phenol from aqueous system include physical adsorption, chemical oxidation, Solvent extraction, aerobic, anaerobic and anoxic biodegradation. The physical adsorption and chemical oxidation processes are not economically viable for treating industrial effluents because of the high phenolic content typically associated with such effluents. The aerobic processes are energy intensive for oxygen transfer and pose severe sludge handling problems.

Application of anaerobic/anoxic biotechnology is now being explored for phenolic wastewater treatment, instead of traditional aerobic processes. Among these processes anoxic ones like denitrification is preferred over others as it has high oxidizing tendency (Gibson, 1984). Some of the advantages of anoxic treatment with nitrate as electron acceptor are higher growth rates and relative insensitivity to inhibitory effects compared to anaerobic microbial consortia and the maintenance of

large levels of soluble electron acceptor thus obviating the requirements of energy intensive aeration systems. Denitrifying microbes have been extensively used for the treatment of nitrate rich wastewaters with methanol, ethanol and acetate as organic carbon sources (Lettinga et al., 1981, Beaubian et al., 1995). Studies on immobilized and granular sludge with such substances have been reported, however there is not much information on the use of granular anoxic sludge for the treatment of phenolic wastewater.

The present investigation is directed to evaluate performance of granular sludge in upflow anoxic sludge blanket (UASB) reactor for the treatment of phenolic wastewater.

2. LITERATURE REVIEW

2.1 General

Phenol (C_6H_5OH) is a monohydroxy derivative of benzene. It has M.P. of 41 ^{o}C and B.P. of 182 ^{o}C. Phenols are made industrially by diazotization of anilines, hydrolysis of aryl halides, alkile fusion of sulfonates. Phenol is colorless and has a distinct and persistent odor. A large fraction of phenol supply goes into the production of plastics (Bakelite) and resins. Phenols are also used in preparing dyes, photographic developers, wood preservatives, flavoring agents, local anesthetics, drugs and perfumes.

Phenol is quite toxic to bacteria when present in high concentration. It has been widely used as a germicide, and other disinfectants have been rated in terms of 'phenol coefficient' i.e., relative disinfecting power of any chemical with respect to phenol. While in a low concentration its biological degradation is possible.

Many industries such as nitrate fertilizers, nitro glyceriene, food processing, meat processing, slaughter house etc. generate high nitrate bearing wastewaters and denitrification, an anoxic dissimilatory process, has good potential for simultaneous removal of organic matter and nitrate from the wastewater. (Deshmukh et al, 1993).

2.2 Sources of Phenol in Wastewater

Phenols are the major toxic pollutants in the wastewaters emanating from industries such as petrochemical, plastics, petroleum refineries, coke and resins manufacturing industries. In general phenolic wastewaters contain phenol as the major

compound of this group with smaller quantities of other phenol derivatives. Table 2.1 shows the phenol concentrations in variety of industrial wastewaters.

TABLE 2.1 : Industrial sources and concentration of phenol *

Industry	Concentration, mg/L
Coking plant :	
Weak ammonia liquor without dephenolization	580 - 10,000
Weak ammonia liquor after dephenolization	4 - 332
Wash oil still wastes	30 - 150
Oil refineries :	
Sour water	80 - 185
General wastewater	10 - 100
APF separator effluent	0.3 - 6.8
Petrochemical :	
Benzene refinery	210
Tar distillation	300
Nitrogen works	250
Orean manufacturing	100 - 150
Plastics factory	600 - 2000
Phenolic resins production	1600
Fiberboard factory	150
Fiberglass manufacturing	40 - 400
Aircraft maintenance	200 - 400

* Adapted from Patterson (1975).

Phenols sometimes occur naturally by decomposition of tannin compounds in swamp waters.

2.3 Effects of Phenol

Phenol that is a toxic substance, causes objectionable taste and odor in water. The medicinal taste of phenol is worsened by chlorination, a common process in water treatment (Gurnham, 1965). The effects of phenol when it is present in a particular water body, are described here (Mckee & Wolf, 1963).

a. Domestic water supplies

The ingestion of concentrated solutions of phenol results in severe pain, renal irritation, shock and possible death. A total dose of 1.5 grams may be fatal.

c. Wildlife watering

According to Heller and pursell (1938), rats that drank water containing phenol from 15 to 1000 mg/l showed no deleterious physiological effects. In test above 7,000 mg/l; growth was stunted and many young rats died at birth.

c. Microorganisms

Phenol is toxic to the microorganisms. Pearson et al. (1980) have shown that phenol concentration of 1000 mg/l inhibited the activity of phenol degrading anaerobic organisms.

d. Fish and other aquatic life

Phenolic compounds affect fish in two ways, first by direct toxic action and second, by imparting a taste to the fish flesh. The toxicity of phenol towards fish flesh increases as dissolved oxygen concentration is diminished.

2.4 Acceptable limits of phenol

Bureau of Indian Standards (BIS) reported different acceptable limits of phenol in different water receiving bodies.

Public water supply : 0.005 mg/L

Surface waters : 1.0 mg/L

Public sewers : 5.0 mg/L

2.5 Treatment Options

The various treatment options available for phenolic wastewaters are physical, physicochemical, chemical, enzymatic and biological processes. These are discussed in brief in this section.

2.5.1 Physical Processes

Kang et al., (1994) reported that the photo-oxidation of wastewater using low pressure Hg lamp irradiation and H_2O_2 as oxidant, is effective for removal of phenol at pH 5 - 8 and at 30 - 50 ^{o}C.

Scheek and Frimmel (1995) reported that phenol removal in water can be done by oxidizing it with U.V. radiation. The irradiation in photoreactor was done by a low pressure mercury lamp.

2.5.2 Physico-Chemical Processes

Adsorption is a separation process in which certain components of a fluid phase are transferred to the surface of a solid adsorbent. These adsorbents are lighly porous material and thus provides a large surface area for adsorption. Activated carbon as an adsorbent was used in various studies.

Najm et al. (1993) used powdered activated carbon (PAC) in upflow floc blanket reactor for adsorption of 2, 4, 6. Trichlorophenol (TCP). PAC adsorption capacity in floc blanket reactor decreased with decreasing influent trichlorophenol concentration. Sorial et al (1993) studied the impact of molecular oxygen for adsorption behaviour of a mixture of

phenolic compounds on fixed bed GAC reactor. Srivastava and Tyagi (1995) prepared a cheap activated carbon from the waste slurry generated in fertilizer plants. This activated carbon was successfully tried for the removal of a variety of substituted phenols like chloro, nitro, trinitrophenols.

Streat et al. (1995) used novel activated carbon prepared by carbonization and subsequent activation of straw and used rubber tyres as well as conventional activated carbons derived from coal, coconut shell and wood. The sorption kinetics of the straw and rubber tyre based carbon were likewise identical to conventional activated carbons.

The activated carbon regeneration requires its burning at a temperature of 600^{o}C under controlled oxygen and moisture conditions thus needing energy. About 5 to 10 % carbon is lost in regeneration. Thus a conversion from one waste to another waste take place.

2.5.3 Chemical treatment

Striolo et al. (1991) utilized wet hydrogen peroxide oxidation process at 120 ^{o}C for treatment of aqueous organic wastewaters. The process was suitable for organic loads upto few gram per litre. The process is energy intensive as temperature of wastewater is to be brought to 120 ^{o}C.

Trapido et al. (1995), studied the ozonation process of some phenols produced at different pH conditions. The results showed that in acidic and neutral media ozonation of phenol is mainly chemical reaction limited and in basic media mass transfer is rate limited.

2.5.4 Enzymatic treatment

Enzymes are proteins that are synthesised by a living cell and act as catalysts in various reactions.

Sun et al. (1992) adopted two step approach for the removal of phenols from aqueous solutions. In the first step, weakly adsorbable phenols are converted to quinones by the enzyme mushroom tyrosinase. The tyrosinase-generated quinones are then chemisorbed on to chitosan, an adsorbent. The mushroom tyrosinase enzyme is quite expensive and can not be used for practical application.

Wada et al. (1993) used mushroom tyrosinase in aqueous solution for removal of phenol. In the treatment with tyrosinase alone, no precipitate was formed but a color change from colorless to dark brown was observed. The difficulty was the necessary need of another adsorbent for removal of color.

2.5.5 Biological Processes

The biological processes are always preferred over chemical and physico-chemical processes. The chemical and physico-chemical processes convert the wastes from one form to another form.

2.5.5.1 Aerobic Processes

Capestany et al. (1977) reported that an activated sludge plant fed phenol at 1000 mg/L and operated with hydraulic retention time of 24 hr. produced an effluent of 0.5 mg/L phenol concentration thus with more than 99 % treatment efficiency.

Rozich et al. (1983) developed a kinetic model for activated sludge treating inhibitory wastewaters and tested experimentally

using phenol as inhibitory carbon source. Monod equation was not found applicable to phenol. Rozich et al. (1985) used various sources of seed populations and batch growth curves were generated using phenol as sole source of carbon.

A completely mixed activated sludge (CMAC) was operated by Pandey and Kaul (1992) at laboratory and pilot scale for treatment of synthetic and live wastewaters. Influent phenol concentration of 1000 mg/L in synthetic phenolic wastewater at 10 h HRT was used for kinetic study. COD removal efficiency of 94 to 96% was found.

These activated sludge processes are energy intensive due to the necessity of an aeration system.

Tyagi et al. (1993) assessed the feasibility of a modified rotating biological contactor (RBC) with polyurethane foam (PUF) attached to the disks as porous support media to biodegrade petroleum refinery wastewater. Ammonia nitrogen and phenol removal were above 99 and 85% respectively.

2.5.5.2 Anaerobic Process

Anaerobic processes are slow process but does not need aeration system unlike activated sludge plants.

2.5.5.2.1 Fermentation

Fermentation is strictly defined as metabolism in which energy is derived from the use of organic compounds as both the electron acceptor and electron donor.

Wang et al. (1989) studied the biodegradability of phenol and substituted phenols in batch phenol-enriched methanogenic cultures. Phenol, at concentrations upto 1400 mg/L was completely degraded to methane and carbon dioxide in 350 h incubation. The

phenol degrading (fermenting) consortium was responsible for the conversion of phenol to acetate and H_2, while methanogenes converts these products to CH_4 and CO_2 (Sheridan et al., 1985).

(1). $C_6H_5OH + 5\ H_2O \longrightarrow 3\ CH_3COOH + 2\ H_2$

(2) $3\ CH_3COOH \longrightarrow 3\ CH_4 + 3\ CO_2$

(3) $2\ H_2 + 0.5\ CO_2 \longrightarrow 0.5\ CH_4 + H_2O$

sum : $C_6H_5OH + 4\ H_2O \longrightarrow 3.5\ CH_4 + 2.5\ CO_2$

Craik et al. (1992) used a continuous feed recycle bioreactor for degradation of phenol by bacteria supported on a bed of GAC & granular biomass. GAC reduced the toxicity of phenol to the microorganisms. The specific phenol degrading activity of biomass supported on GAC was inferior to that of granular biomass and suspended biomass.

The expanded bed anaerobic GAC reactor, operating with GAC replacement was used to treat toxic wastes (Nakhala and Suidan, 1992). GAC absorbed the toxic pollutant as well as provided surface for microbial attachment. The problem with using GAC was its exhaustible adsorptive capacity, thus demanding replacement of exhausted GAC with fresh one for long term operations.

2.5.5.2.2 Anoxic Sulfate Reduction

Sulfate can serve as a terminal electron acceptor under anaerobic conditions. However, only a few microbial systems have been observed to degrade aromatic compounds by this means. Degradation of Benzoates coupled with sulfate reduction has been demonstrated in pure cultures (Widdel et al. 1983) and in cocultures (Balba and Evans, 1980).

2.5.5.2.3 Anoxic Denitrification

In conventional denitrification processes, denitrification is aimed to remove organic compounds from the wastewater (Gayle et al. 1989; Dahab and Lee, 1988).

Recently anoxic denitrification processes are attracting wide attention due to some of the advantages of these processes over aerobic and anaerobic methods.

1. No energy requirement while aerobic processes are highly energy intensive.
2. The denitrifying mixed cultures are relatively insensitive to inhibitory effects compared to anaerobic processes.
3. The denitrifying bacteria exhibit higher growth rates.
4. Setting the anaerobic conditions required for denitrification is unproblematic, because the denitrifying mixed cultures tolerate small amount of dissolved oxygen.

In the denitrification process, nitrate is reduced to molecular nitrogen via several intermediate stages. Focht and Chang (1975) proposed the following path

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

Denitrification is a process that takes place under anaerobic conditions, where the links of above mentioned reaction chain act as electron acceptors. Each step in the denitrification process is catalyzed by a separate enzyme system. Nitrate is used preferentially over nitrite. Even though both nitrate and nitrite reductase are present.

Denitrification is considered to be a *heterotrophic process*

conducted by microorganisms that require a reduced organic substrate for energy and cell synthesis. Several workers used phenols as an electron donor (Hu and Sheih, 1987 ; Deshmukh et al. 1993). The following stoichiometric relationship have been formulated.

$$5C_6H_5OH + 28\ NO_3^- \longrightarrow 14\ N_2 + 30\ CO_2 + H_2O + 28\ OH^-$$

Denitrification can also be accomplished by *autotrophic bacteria* which can use hydrogen (Kurt et al., 1987) or various reduced sulfur compounds (Barrenstein et al., 1986) as energy sources. The following stoichiometric relationships have been reported.

$$2\ NO_3^- + 5\ H_2 \longrightarrow N_2 + 4\ H_2O + 2\ OH^-$$

$$5\ S^{--} + 8\ NO_3^- + 8\ H^+ \longrightarrow 5\ SO_4^{--} + 4\ N_2 + 4\ H_2O$$

There is a great variety of facultative anaerobes that are capable of carrying on denitrification. Some of these are Bacillus, Pseudomonas, Alcalaigenes, Micrococeus, Spirillum, Moracella, Hyphomicrobium, Xanatnomonas, Glucanobacter etc.

Hu and Shieh, (1987) reported investigations on the degradation of certain monocyclic aromatic compounds including phenol, induced by the biofilms under anoxic conditions, with potassium nitrate as the electron acceptor. They used upflow biofilter with glass beads as immobilisation medium. Ten day after the inoculation, 85 % total organic carbon and 65 % feed NO_3-N was removed at an HRT of 2.5 h.

Godbole and Chakrabarty, (1991) found an upflow anoxic

fixed film-fixed bed (UAFFFB) reactor to be an effective system for the continuous long term biodegradation of Phenol, resorcinol catechol, cyclohaxonal and cyclohexanone. The study highlighted the biochemical specificity acquired by a seed when exposed to different substrates for acclimation. For COD loading of 0.264 to 0.282 g COD/L-d, 42 to 71% phenol removal was found after 24 h.

Deshmukh et al. (1993) employed resorcinol, a phenol derivative as electron donor in UAFFFB denitrification column. Performance evaluation studies indicated excellent resorcinol and nitrate removal under anoxic conditions. For COD loading of 6 g COD/L-d and HRT of 8 h or more, it was found that COD and nitrate removal efficiency was more than 90 % .

3. SCOPE OF THE STUDY

The production of phenolic wastewater is increasing due to great reliance on coal for energy and base organic chemicals, thus there is need to develop an efficient system for phenolic wastewater treatment. From the preceeding chapters, it is evident that toxic organic compounds like phenol, can serve as source of organic carbon for heterotrophic denitrifying bacteria and thus can be detoxified by anoxic biodegradation. Although there are some reports on anoxic biofilm reactors, there is scantly information available on the use of anoxic granular sludge for phenol removal. The present investigation was, thus, directed to detoxify the hazardous phenolic effluents using anoxic granular sludge and was undertaken on the following lines.

1. Development of phenolytic denitrifying granular biomass.
2. Design and fabrication of a laboratory scale upflow anoxic sludge blanket (UASB) reactor.
3. The evaluation of performance of UASB reactor at different influent phenol concentrations in terms of phenol, NO_3-N, total & soluble COD removals.
4. Determination of the maximum space loading rate of toxic phenolic effluents that can be sustained and degraded by anoxic granular sludge.
5. Determination of the maximum specific sludge loading rate which microbes can withstand with efficient removal of phenol.
6. Determination of toxic influent phenol concentration to the phenolytic denitrifying granular biomass.

4. EXPERIMENTAL METHODOLOGY

In the present investigation, a mixed culture of phenolytic denitrifiers were employed to remove phenols from the wastewater in a Upflow Anoxic Sludge Blanket (UASB) reactor configuration. The present chapter deals with the experimental methodology used for the developing the phenolytic denitrifiers and evaluate their performance in UASB reactor.

4.1 MICROBIAL CULTURE

Granular anoxic phenolytic sludge was developed in laboratory using garden soil as inoculum by Iyengar, (1995). The culture medium consisted of 500 mg of glucose, 100 mg of phenol and enough Sodium Nitrate (to maintain a ratio of Organic C/NO_3-N of 1.2) and 50 ml of nutrient solution (composition presented in Table 4.1) to one litre of tap water. 2 g garden soil was added to the culture medium in a 2 litre conical flask and kept at 25^{o}C in the incubator (Remi, India). After 2 days the sedimented soil was removed by decanting the supernatant into another flask. The reactor was maintained at 3 days hydraulic retention (HRT) time and was operated in sequential batch mode. The bacterial growth was indicated by increased turbidity of culture medium. The rate of phenol removal was not high initially but increased with time. When phenol removal attained was 70 to 80 %, glucose in the culture medium was decreased gradually with simultaneous increase in phenol as carbon source for enrichment of phenolytic organisms. Flocs were observed after one month and phenolytic granules appeared after three months of operation with 300 mg of phenol. At this stage HRT was gradually decreased and maintained

at 1.2 d. The phenol concentration was gradually increased to 500 mg/L (Iyengar, 1995). The culture was of a mixed type with predominance of *'Bacillus circulans'* microflora as identified by Microbial Type Culture Collection (MTTC), Chandigarh.

TABLE 4.1 Composition of Nutrient Solution

Substance	Concentration (g/l)
Potassium Dihydrogen Orthophosphate (KH_2PO_4)	4.0
Manganese Chloride ($MnCl_2$)	0.04
Sodium Molybdate ($NaMoO_4$)	0.02
Yeast Extract	0.4

4.2 Experimental set up

The schematic figure of experimental setup having UASB is presented in Figure 4.1.

4.2.1 The Upflow Anoxic Sludge Blanket (UASB) reactor

A UASB reactor was fabricated from a plexiglass (acrylic) column with an internal diameter of 57 mm and 750 mm height. The volume of the reactor is 1.35 litre. For proper distribution of feed, a circular distributor having 3 holes of 2.5 mm diameter each was used at bottom of the reactor. The distributor was connected to feed pump by tubes.

One of the salient feature of the UASB reactor is the gas-liquid-solid separation (GLSS) device in upper part of the reactor. It consisted of a acrylic deflector ring, on top of

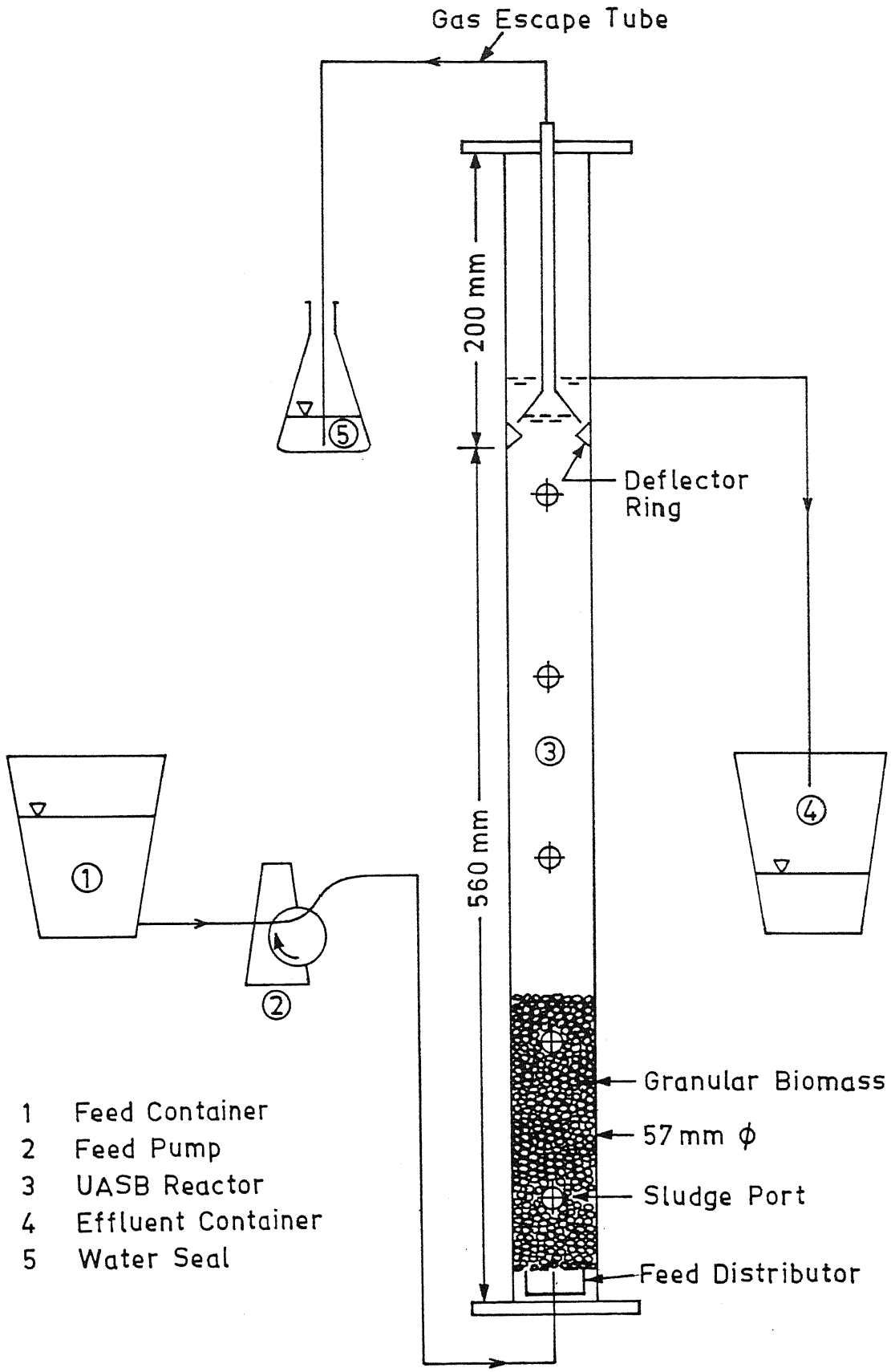


Fig. 4.1. Schematic Diagram of Experimental Setup of Upflow Anoxic Sludge Blanket (UASB) Reactor.

which was placed an inverted plastic cone of 45 mm O.D. The deflector ring of 37 mm I.D. & 20 mm height was tapered at 45^{o} angle to the inner edge at both sides and was placed at 560 mm height. An aperture (a gap between slant surface of gas domeand deflector beam) of 2 mm and an overlap (a radial distance between gas dome O.D. and deflector beam's I.D.) of 4 mm was provided. The 55^{o} angled cone wall was designed to aid settling of the sludge.

Sludge withdrawal ports at different heights were made for periodic removal and analysis of sludge in the reactor. Two effluent ports opposite to each other were provided at a level of 10 mm above the slant surface of gas dome.

4.2.2 Feed Pump

For delivering continuous constant feed to the reactor a peristaltic pump (TRIS, ISCO, Inc, USA) was used. Silicon tubes of following three sizes were used for the pump. (i) 7.5 mm O.D. & 1.5 mm thickness allowing max flow of 780 ml/hr; (ii) 7 mm O.D. & 2.5 mm thickness allowing max flow of 300 ml/hr; (iii) 6 mm O.D. & 2 mm thickness allowing max. flow of 240 ml/hr.

4.2.3 Feed and Effluent Container

Plastic buckets of 12 litre volume used for the feed & effluent collection purposes. A tube, fixed at the bottom opening of the feed container was connected to the peristaltic pump.

4.2.4 Water Seal

The gas escape tube from the reactor was dipped in the water seal system. To maintain a particular level of gas

liquid surfaces inside the gas dome its difference with the effluent discharge level must be given as head in the water seal. A beaker filled with water upto 30 mm level was used for this purpose.

The experimental set up was put into operation in July'95. The granular sludge from sequential batch reactor was transferred to the upflow anoxic sludge blanket reactor. For start up of the reactor, 750 mg/L of phenol with appropriate quantity of nitrate and nutrient solution (composition presented in Table 4.1) was supplied in the influent at 24 h HRT. The study was done at different concentrations of phenol ranging 600 mg/L to 1100 mg/L and different HRTs ranging from 24 h to 2 h. The entire set up was kept inside the incubator (Remi, India) at 30 ^{0}C in Dec.'95 due to steep fall in ambient temperature.

4.3. Methods of Study

4.3.1 Studies on liquid phase

Synthetic phenolic wastewater was prepared and was used as feed for the phenolic denitrifiers. Influent and effluent samples from the reactor were analyzed for phenol, NO_3-N, NO_2-N, COD and TSS concentrations.

4.3.2 Studies on solid phase

The important part of the reactor is the phenolytic biomass. Samples at different sludge withdrawal ports were collected and analyzed for total suspended solids (TSS) & volatile suspended solids (VSS) concentrations. Data was collected to make a sludge profile (a plot of sludge concentration vs height of reactor). The microscopic examination of granular sludge was carried out by Scanning Electron

Microscope (Jeol JSM-840A, Japan).

4.3.3 Studies on gas phase

Various gases are evolved during the metabolic processes of organisms. The gas sample was collected in 2 ml syringe and was analyzed for its composition by Gas Chromatograph (NUCON -5700, India) with thermal conductivity detector, using Carbosphere column. Helium gas was used as a carrier gas in the Chromatograph.

4.4 Analytical Techniques

4.4.1 Phenol

4-Aminoantipyrene direct photometric method as described in *standard methods (1989)*, was used for the estimation of phenol in effluent. Phenol reacts with 4-Aminoantipyrene at a pH of 7.9 $\pm$ 0.1 in the presence of potassium ferricyanide to form a colered dye. The absorbance of dye is measured at 510 nm using Visible Spectrophotometer (Systronics, India).

4.4.2 Nitrate-Nitrogen

Ultraviolet spectrophotometer method as described in *standard methods (1976)*, was used for NO_3-N estimation. Nitrate absorbs light at 220 nm. Because dissolved organic matter also absorb at 220 nm and nitrate does not absorb at 275 nm, a second measurement is made at 275 nm to correct the nitrate value. 2 times absorbance at 275 nm is subtracted from that at 220 nm for correction. The absorbances were measured by UV Visible Spectrophotometer (UV-160A, Shimadzu, Japan). Acidification with 1N HCl was adapted to prevent interference from OH^- & CO_3^- ions.

4.4.3 Nitrite-Nitrogen

Nitrite-nitrogen in the effluent was estimated by

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colrimetric method, as described in *standard methods (1965)*. Nitrite concentration is determined through the formation of reddish purple azo-dye produced ar pH 2.0 to 2.5 by the coupling of sulfanilamide with N-(1-napthyl)-ethyldiamine dihydrochloride. Visible spectrophotometer (Systronics, India) was employed for measuring the dyes absorbance at 520 nm.

4.4.4 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) test is widely used as a means of measuring organic strength of wastewater. This test allows measurement of a waste in terms of total quantity of oxygen required for oxidizing organic carbon to carbon dioxide and water. The reaction occurring is as follows :

$$\text{Organic Matter } (C_aH_bO_c) + Cr_2O_7^{--} + H^+ \longrightarrow Cr^{3+} + CO_2 + H_2O$$

Potassium dichromate is used as an oxidizing agent in acidic medium and in presence of silver sulfate as a catalyst. The chloride interference is eliminated by adding Mercuric sulfate (Hg_2SO_4). The sample is refluxed for 2 h and after digestion the remaining unused $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and thus COD. Influent and effluent samples for COD were centrifuged at 6000 g (Remi, India) for 10 min. Both centrifuged (soluble) and uncentrifuged (total) samples were analyzed for COD using open reflux method (standard methods, 1989).

4.4.5 Total Suspended Solids (TSS)

A well mixed sample is filtered through a weighed Watman-1 filter paper and the residue retained on filter is dried

to a constant weight at 103 to 105 ^{o}C. The increase in weight of filter represents the total suspended solids (TSS). The determination was done as per *standard methods (1989)*.

4.4.6 Volatile Suspended Solids (VSS)

Volatile suspended solids analysis was done for solid phase to measure the biomass concentration in the reactor. The sample was centrifuged at 6000 g for 10 min. Settled solids were dried at 103 ^{o}C to 105 ^{o}C and weighed to find TSS. This residue is ignited to constant weight at 550 ^{o}C $\pm$ 50 ^{o}C in the muffle furnace. The weight loss on ignition is volatile suspended solids (VSS). The determination of VSS was done as per *standard methods (1989)*.

5. RESULTS & DISCUSSION

Biological denitrification has been extensively used for the treatment of nitrogen rich wastewater as well as effluent from aerobic treatment units. Organic carbon source in the form of acetate, methanol or ethanol is provided for hetrotrophic denitrification whereas oxidizable sulfur and hydrogen are the electron donors for autotrophic denitrification. Recently anoxic denitrification as a method for organic carbon removal has been attracting wide attention due to several advantages of this process over aerobic and anaerobic biological processes (Gayle et al, 1989). Earlier studies had shown that denitrifiers can utilize phenol as the sole organic carbon source (Aftring & Taylor, 1981 ; Hu & Sheih, 1987). The treatment of Phenolic wastewater using immobilized microbial cells under anoxic conditions have been reported (Hu & sheih,1987, Deshmukh et al.,1993). However, no study has been conducted on use of granular sludge. Thus the objective of the present investigation was to evaluate the performance of anoxic denitrifying granular sludge for the treatment of phenolic wastewaters.

5.1 Start up of the Reactor

Granular anoxic sludge development was carried out in a separate sequential batch reactor from enriched phenolytic heterogeneous bacterial culture which was isolated from soil (section 4.1.). Granules were observed only after three months in the once fed sequential batch reactor which was maintained on the synthetic wastewater containing 300 mg/L phenol with required amounts of nitrate and the nutrients solution at an hydraulic

retention time (HRT) of 1.2 days. Influent phenol concentration was then increased stepwise up to 750 mg/L and maintained at this level till the granules were transferred to a UASB reactor . The initial volatile suspended solids (VSS) in the UASB reactor was 38 g with sludge blanket height of 190 mm. The reactor was started by continuously feeding the synthetic phenolic wastewater with a composition shown in Table 5.1 at the HRT of 24 h. The initial space loading rate (S.L.R.) and sludge loading rate (Sl.L.R.) were 1.675 g COD/L-d and 0.06 g COD/g VSS-d respectively. Initially UASB reactor was operated at ambient temperatures which was later transferred to an incubator maintained at 30^{o}C.

TABLE 5.1. Composition of synthetic phenolic wastewater

Substance	Concentration
Phenol, mg/L	750
Sodium Nitrate, mg/L	3030
Nutrient*, ml/L	30

* Nutrient composition is presented in Table 4.1.

5.2 Overall performance of UASB reactor

UASB reactor was continuously operated for a period of 233 days. Figure 5.1 illustrates the variation in space loading rate and phenol removal efficiencies during this entire time period . High removal efficiency was observed just after the start up of UASB reactor . Influent phenol concentration was increased stepwise maintaining the HRT of 12 h from 5th to 24th day. Upto

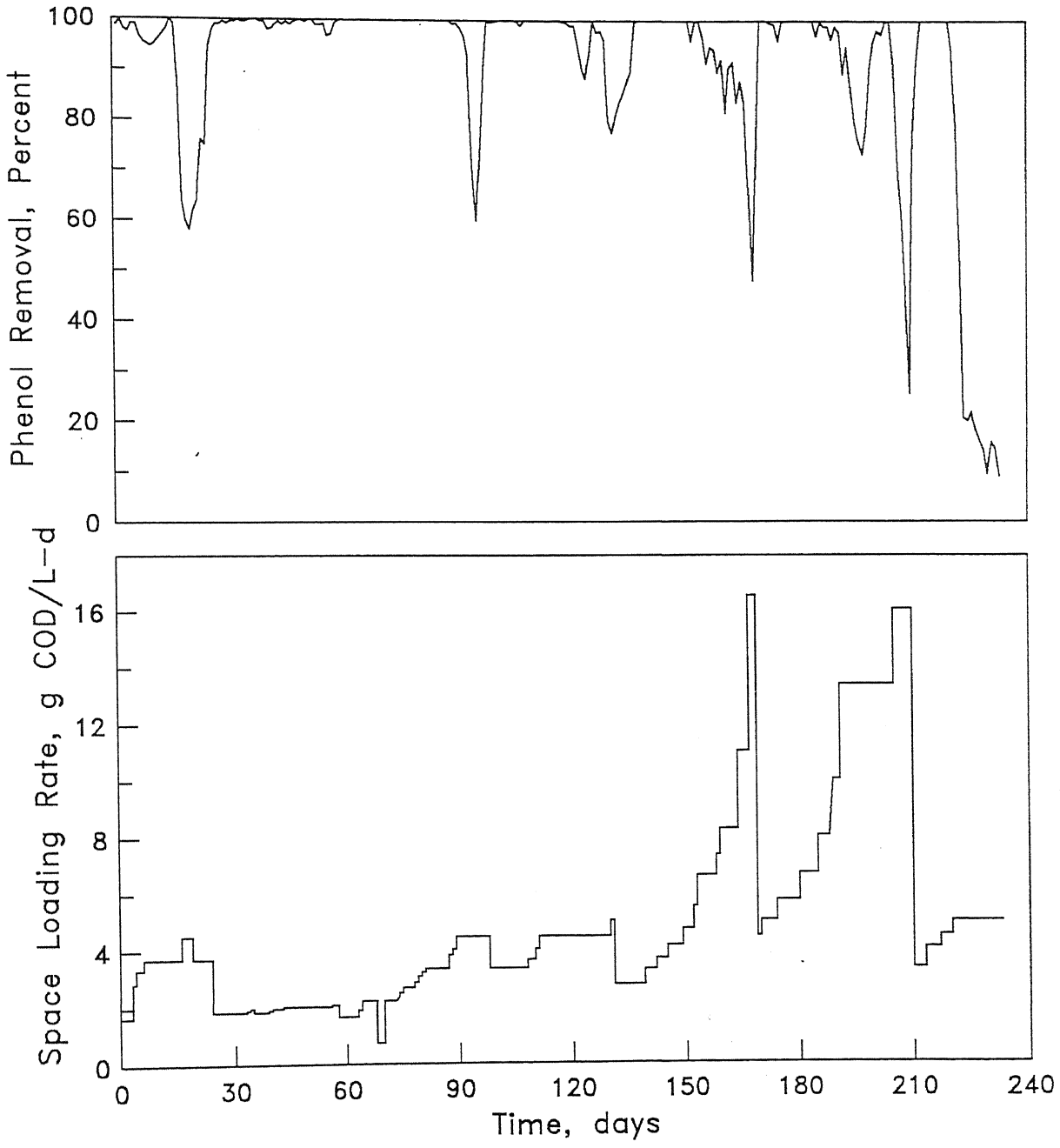


Fig. 5.1. Variations of Space Loading Rate and Phenol Removal with time

an influent phenol concentration of 825 mg/L which is equivalent to COD space loading rate of 3.7 g COD/L-d, Phenol removal efficiency was more than 98 % . However on the 17th day of operation, when influent phenol concentration was increased to 1000 mg/L corresponding to an S.L.R. of 4.5 g COD/L-d , a drastic reduction in phenol removal was observed. It could be due to toxicity of phenol to the microbes. At this stage phenol concentration was decreased in steps to 750 mg/L (S.L.R. of 2 g COD/L-d), which resulted in the recovery of microbes and hence the reactor performance as shown by phenol removal efficiency of more than 98 % . This efficiency was continued to be maintained even when S.L.R. was increased to 4 g COD/L-d.

Since the ambient temperature decreased from the initial 35^{o}C to 17^{o}C during 4 months of operations , the reactor was transferred into an incubator maintained at 30 ^{o}C . From 129 th day, influent phenol concentration of 600 mg/L (COD equivalent 1380 mg/L) was maintained. S.L.R. was increased by decreasing the HRT stepwise from 12 to 2 h over a period of 40 days. For the decrease of HRT from 12 to 7 h with corresponding S.L.R. of 4.73 g COD/L-d, phenol was undetectable in the effluent. Treatment efficiency of above 80 % could be achieved upto a HRT of 3 h (S.L.R. of 11 g COD/L-d). However, when the S.L.R. was increased to 16.56 g COD/L-d(2 h HRT), a drammatic increase in effluent phenol concentration was observed indicating the failure of system which was then operated at increased HRT of 9 h for its revival.

After the revival, the reactor was fed with an increased

phenol concentration of 750 mg/L maintaining the HRT of 8 h from 171st day. Corresponding to this, the phenol removals were of the order of 98 % . The HRT was decreased gradually from 8 h to 2.5 h. At 3 h HRT, a decrease in efficiency was initially observed followed by improved performance. Phenol removal was more than 99 % corresponding to 80 hydraulic turnovers at this HRT. This can probably be attributable to enhanced rate of gas production resulting in the efficient mixing between biomass and the substrate. However, further decrease in HRT to 2.5 h (S.L.R. of 16.1 g COD/L-d) resulted in failure of the system. Again HRT was increased to 24 h for the system to recover.

The last phase of the study was to determine the toxic concentration of phenol to the biomass, maintained at a high HRT of 12 h. The phenol concentration was gradually increased from 750 to 1100 mg/L. Upto 1000 mg/L, phenol was undetectable in the effluent indicating a very high efficiency. However beyond 1000 mg/L i.e., at 1100 mg/L phenol concentration even though the S.L.R. was 5.0 g COD/L-d only, the phenol removal was reduced to 20 % which was further decreased when maintained at the same feed conditions. This was slightly different from the observation made at the initial stage of this study (upto 20 days) where at an influent phenol concentration of 1000 mg/L, a marked decrease in removal efficiency was noted even with 24 h HRT. This could be due to the increased granular biomass concentration at the end of this study which was 72 g as against initial 38 g.

5.3. Analysis of Steady State Reactor Performance

As discussed, the reactor was shifted to an incubator for

maintaining the biomass at a constant temperature of 30 ^{o}C. Initially the reactor was fed with 600 mg/L phenol at 12 h HRT which was gradually decreased to 2 h. At each of these HRTs, the effluent concentration of phenol, COD (total & soluble), nitrate and nitrite were determined after attainment of steady state. The effects of increased influent phenol concentration (750 mg/L) on the performance of the reactor was also studied. The analysis of performance with respect to space loading rate (S.L.R.) as well as sludge loading rate (Sl.L.R.) are presented below.

5.3.1 Effect of Space Loading Rate (S.L.R.) on Reactor Performance

The reactor performance as assessed on the basis of phenol, NO_3-N, COD removals and effluent NO_2-N and TSS concentrations are presented in Figures 5.2 to 5.4 for the initial phenol concentrations of 600 and 750 mg/L.

At a S.L.R. equal to or less than 5 g COD/L-d the phenol, nitrate, and total & soluble COD removals were almost 100 % irrespective of initial phenol concentrations. An increase in S.L.R. upto 13.6 g COD/L-d also gave nearly 100 % removals for a phenol concentration of 750 mg/L (Figure 5.2). An increase beyond this drastically decreased the removal efficiency. However, the performance of granular sludge was better than that reported earlier by Deshmukh et al (1993) with fixed film reactor which showed 77 % removal efficiency at S.L.R. of 7 g COD/L-d. A lower phenol removal efficiency (Figure 5.2) was observed upto S.L.R. of 11 g COD/L-d, when the initial phenol concentration was decreased to 600 mg/L. Further increase in S.L.R. resulted

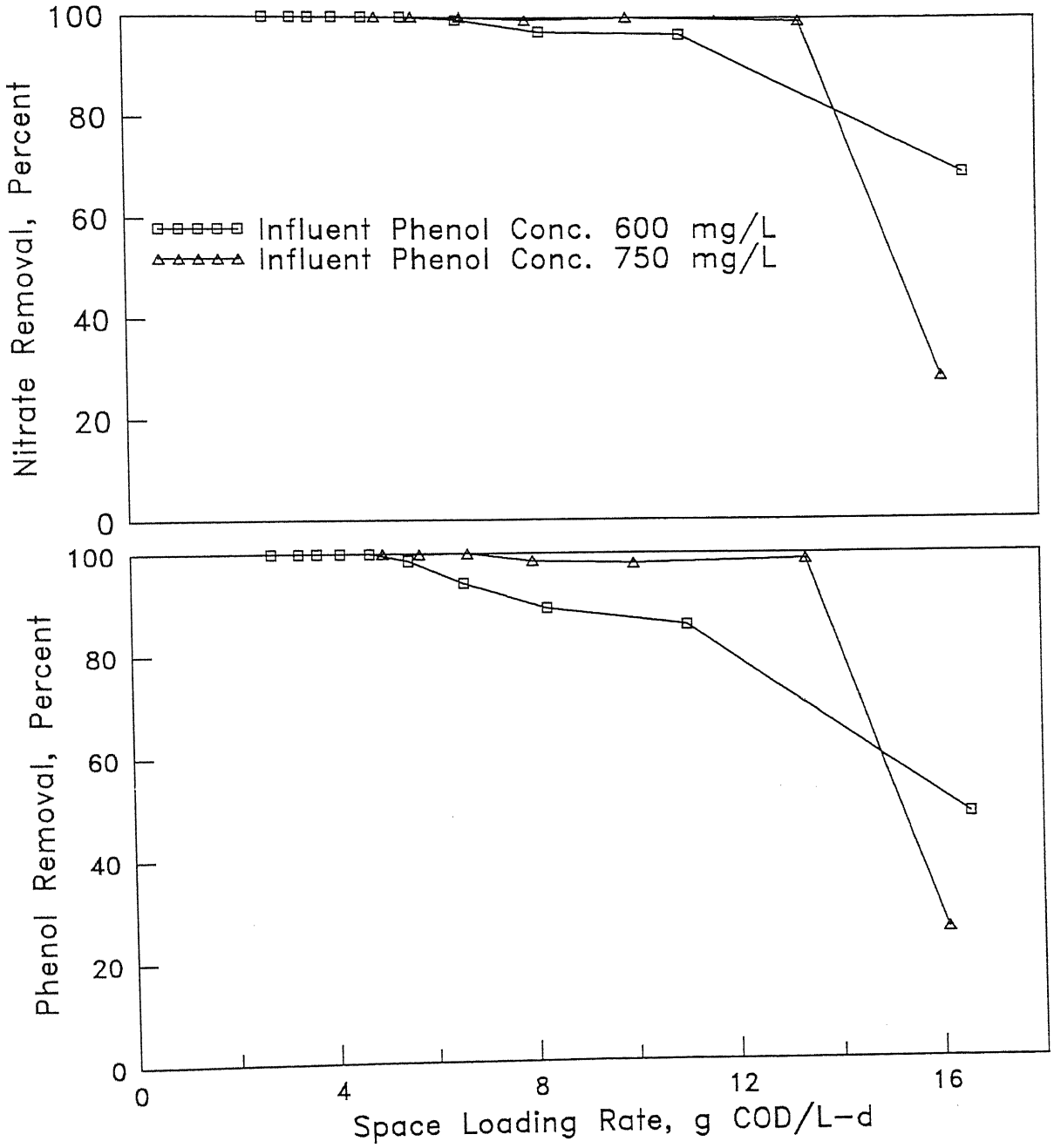


Fig. 5.2. Phenol & Nitrate Removals as a function of Space Loading Rate

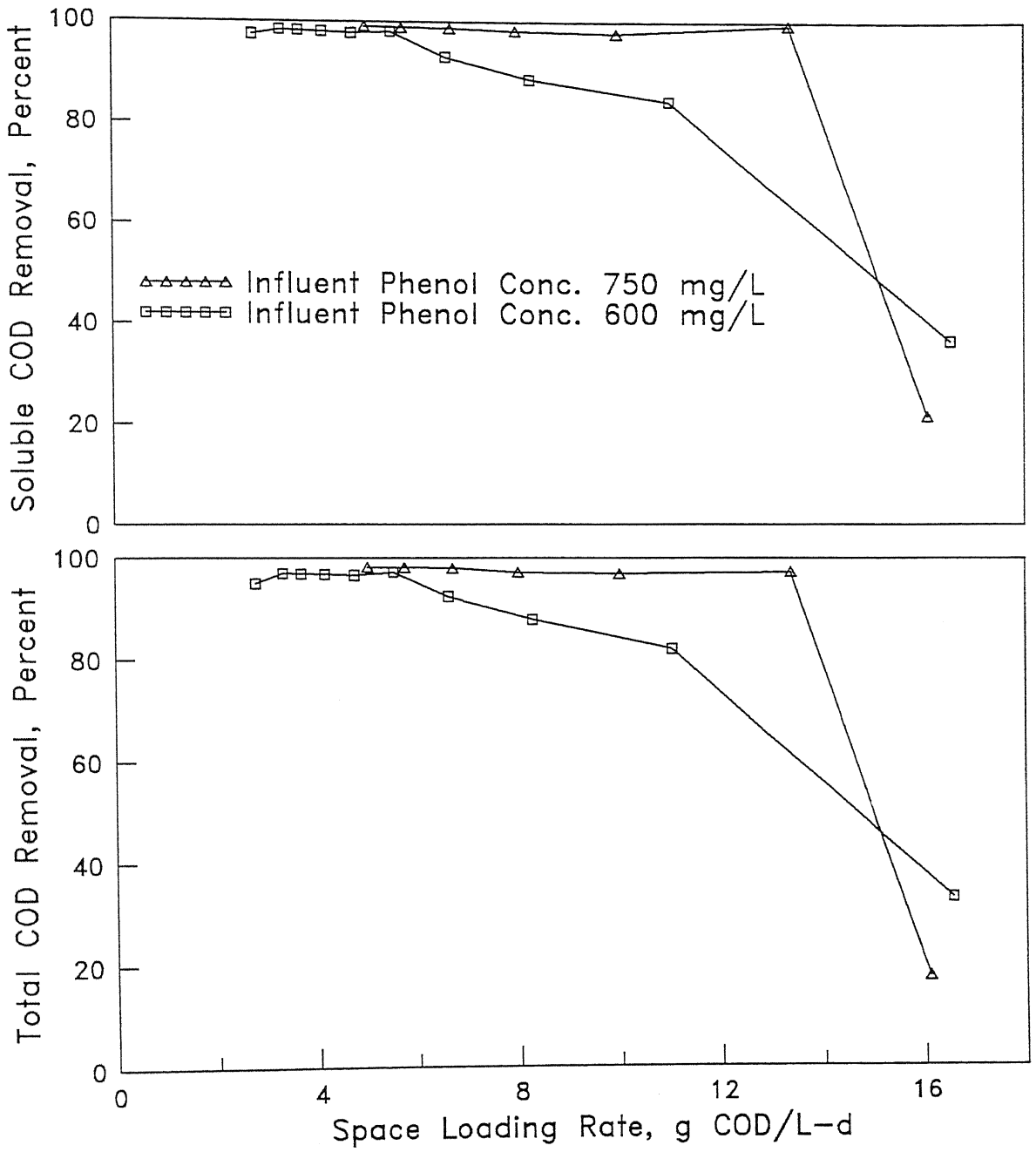


Fig.5.3. Total & Soluble COD Removals as a function of Space Loading Rate

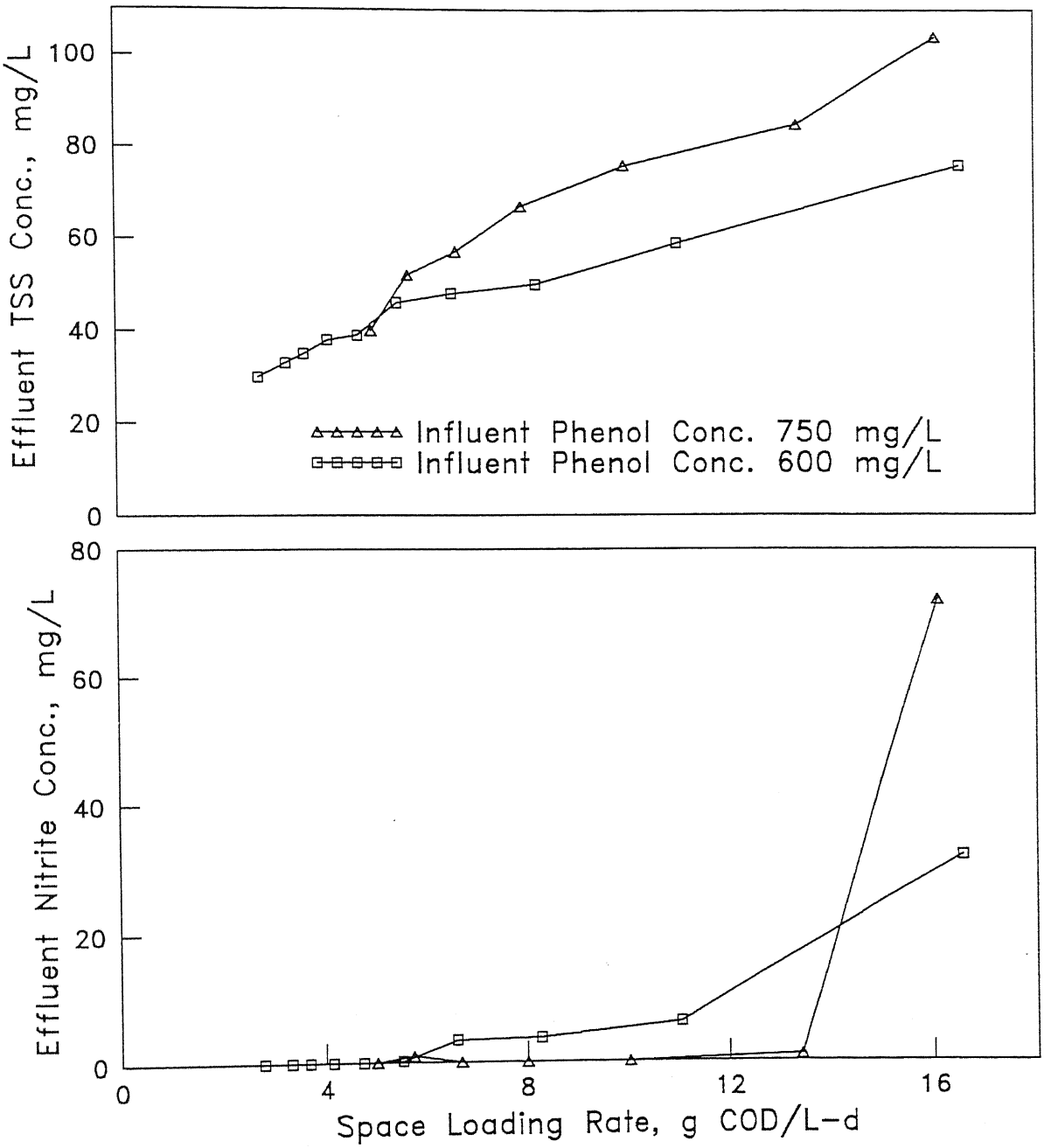


Fig. 5.4. Effluent Nitrite & TSS Conc.'s as a function of Space Loading Rate

in faster decrease in phenol removal efficiency.

It is, thus, obvious that performance of reactor with a higher influent concentration (750 mg/L) is better than that at 600 mg/L phenol concentration. This could be attributed to enhanced diffusion of phenol to the granular surface of biomass due to higher concentration gradient and possibly higher rate of its utilization.

Figure 5.4 illustrates the variations of effluent TSS and nitrite concentration w.r.t. S.L.R. Upto 5.5 g COD/L-d, nitrite was not detectable in the effluent. For feed with phenol of 600 mg/L, when S.L.R. was increased further, nitrite concentration of effluent increased gradually. In case of of 750 mg/L of phenol, nitrite was undetectable upto the S.L.R. of 13.6 g COD/L-d. When S.L.R. was further increased to 16.1 g COD/L-d, nitrite concentration of effluent shot up to 72 mg/L. There appears to be a correlation between nitrite accumulation and phenol, COD and nitrate removals comparing the Figures 5.2 to 5.4. Nitrate accepts electrons produced during the microbial degradation and is ultimately converted to N_2 gas with nitrite as one of the intermediates. The accumulation of nitrite indicates the inhibition of enzyme, nitrite reductase, resulting in nonachievement of complete denitrification.

Total suspended solids (TSS) increased with increase in S.L.R. The UASB reactor produced effluent conforming to TSS standards of 50 mg/L below a S.L.R. of 6.8 g COD/L-d for influent phenol concentration of an 750 mg/L and 11 g COD/L-d for phenol of 600 mg/L. A gradual increase in TSS beyond these S.L.R.

values indicates escape of lighter flocculant puffy biomass from the reactor.

5.3.2 Effect of Sludge Loading Rate (Sl.L.R.) on reactor performance

The effects of sludge loading rate (Sl.L.R.) on reactor performance are presented in Figures 5.5 to 5.7. Treatment efficiencies in terms of phenol and COD removal of above 80 % could be achieved upto an Sl.L.R. of 0.26 g COD/g VSS-d. Further increment in Sl.L.R. to 0.30 g COD/g VSS-d resulted in a drammatic increase in effluent COD, phenol, NO_3-N, NO_2-N, and TSS, indicating the failure.

The treatment efficiencies for phenol concentration of 750 mg/L was observed to be better than that at 600 mg/L as in the case of S.L.R.. The stoichiometry for organic carbon and NO_3-N removal was calculated to be equal to 1.15 : 1. This is similar to the ratio observed with easily assimable organic carbon sources such as methanol and ethanol (Beaubien et al., 1995). The yield of biomass was 0.11 g VSS/g phenol which was in agreement with the study of Hu & Sheih (1989). The maximum specific removal rate was 0.23 g COD/g VSS-d.

5.4 Effect of Phenol Concentration on Reactor Performance at Constant HRT

Phenol concentration of the influent was increased upto 1100 mg/L maintaining HRT of 12 h during the last phase of study i.e., from 211 th day. Variations in phenol, nitrate and COD removals with increase in phenol concentrations are illustrated in Figure 5.8., while variations of effluent nitrite and TSS concentration are presented in Figure 5.9.

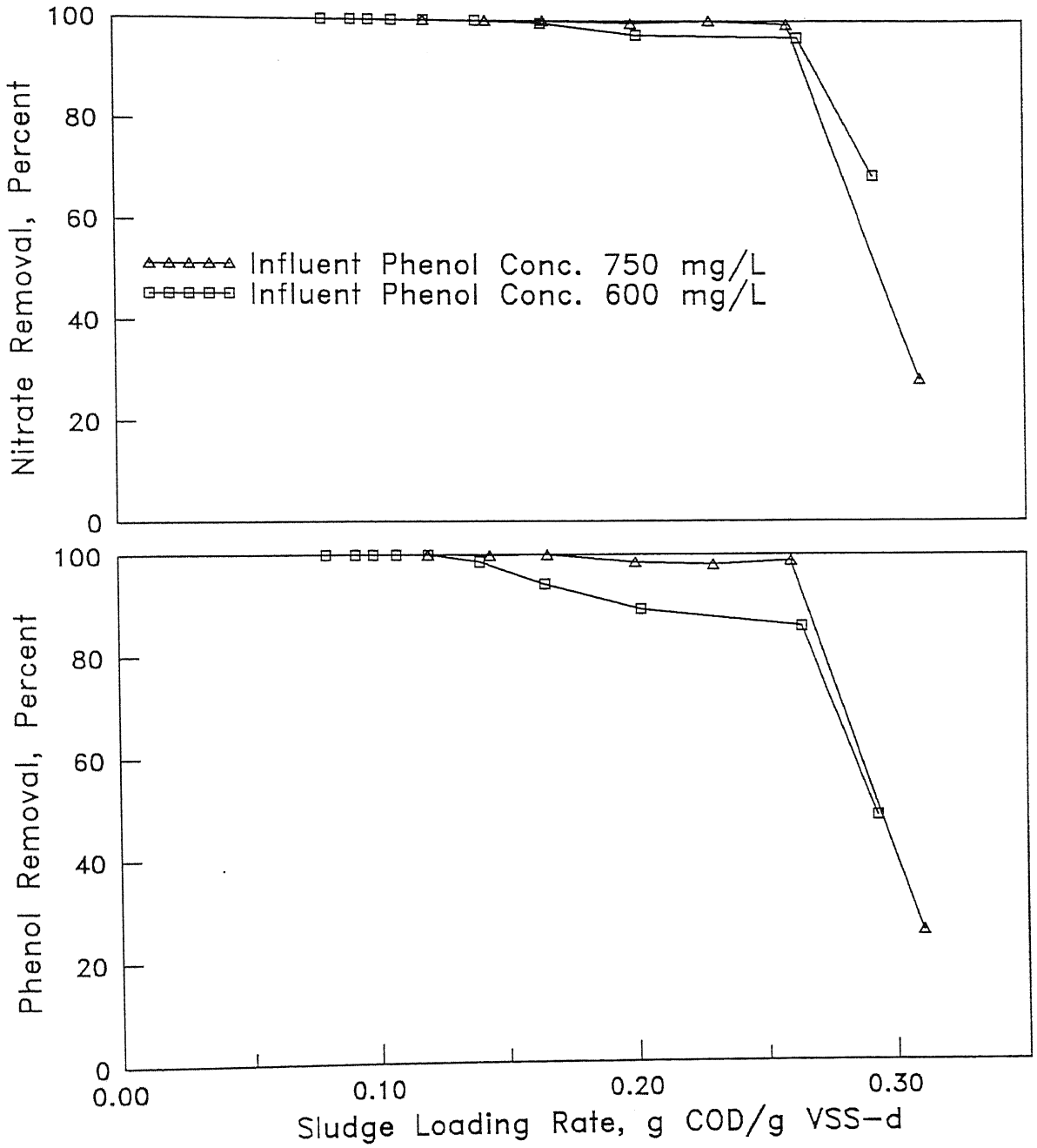


Fig. 5.5. Phenol & Nitrate Removals as a function of Sludge Loading Rate

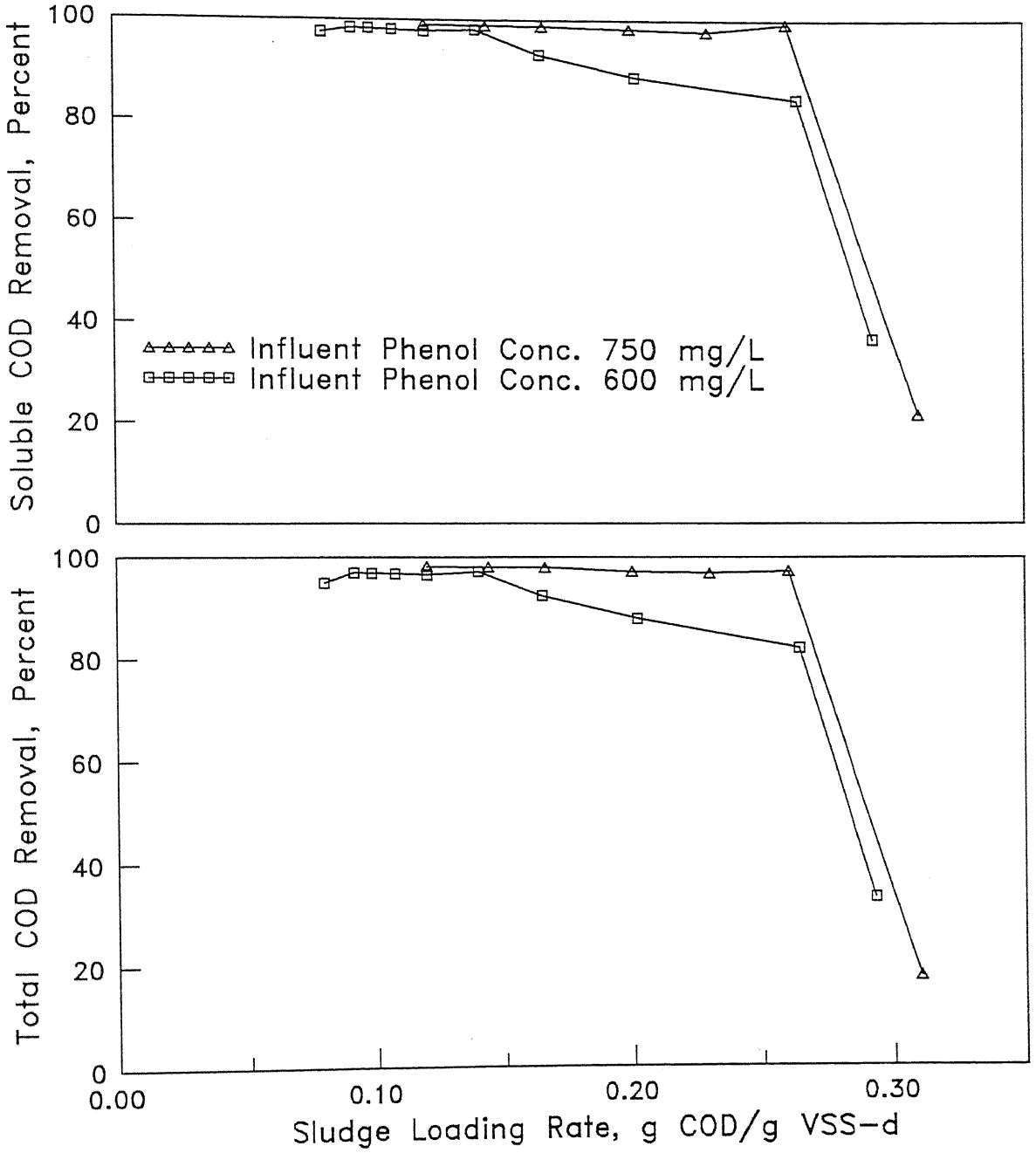


Fig. 5.6. Total & Soluble COD Removals as a function of Sludge Loading Rate

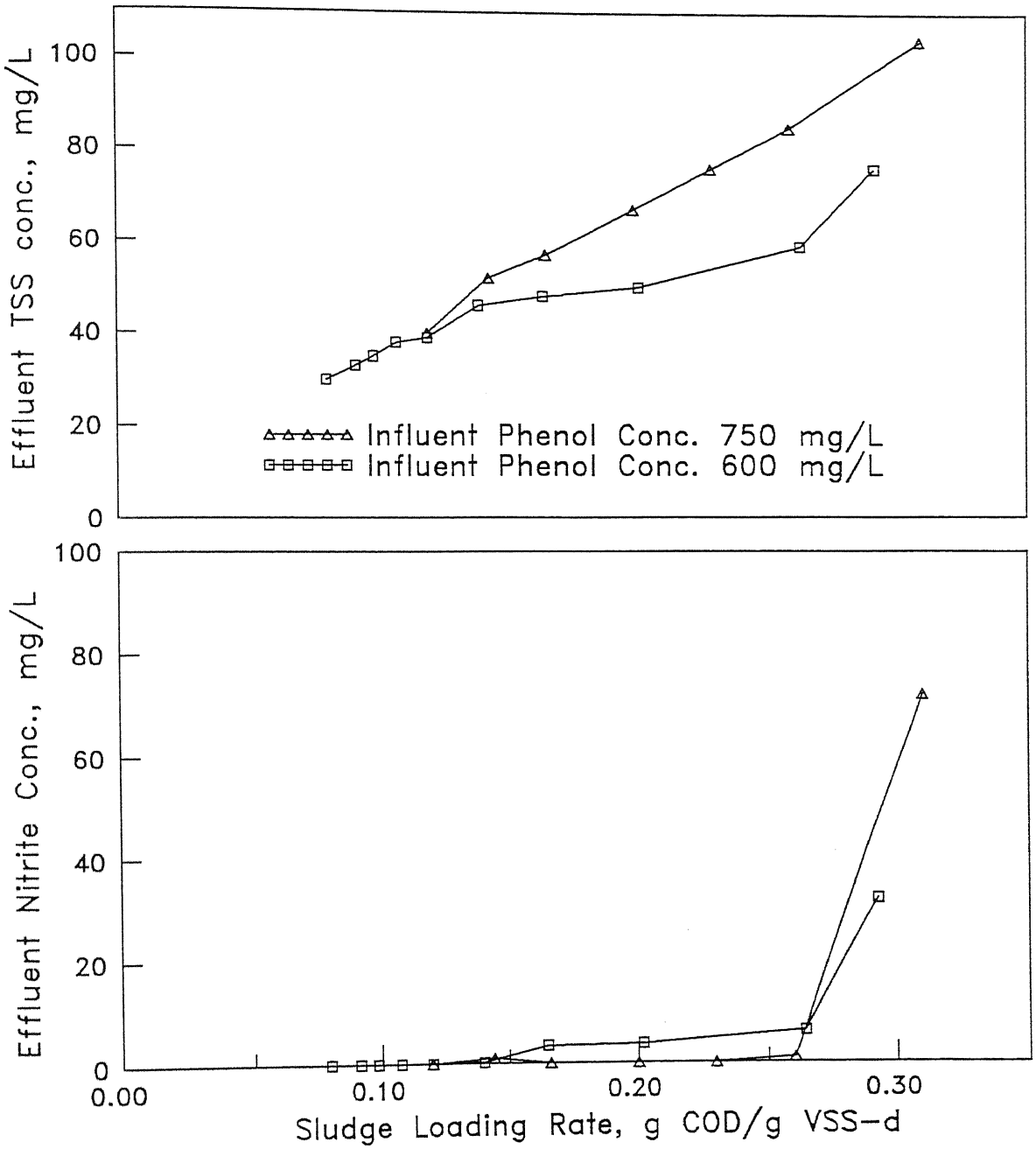


Fig. 5.7. Effluent Nitrite & TSS Conc.'s as a function of Sludge Loading Rate

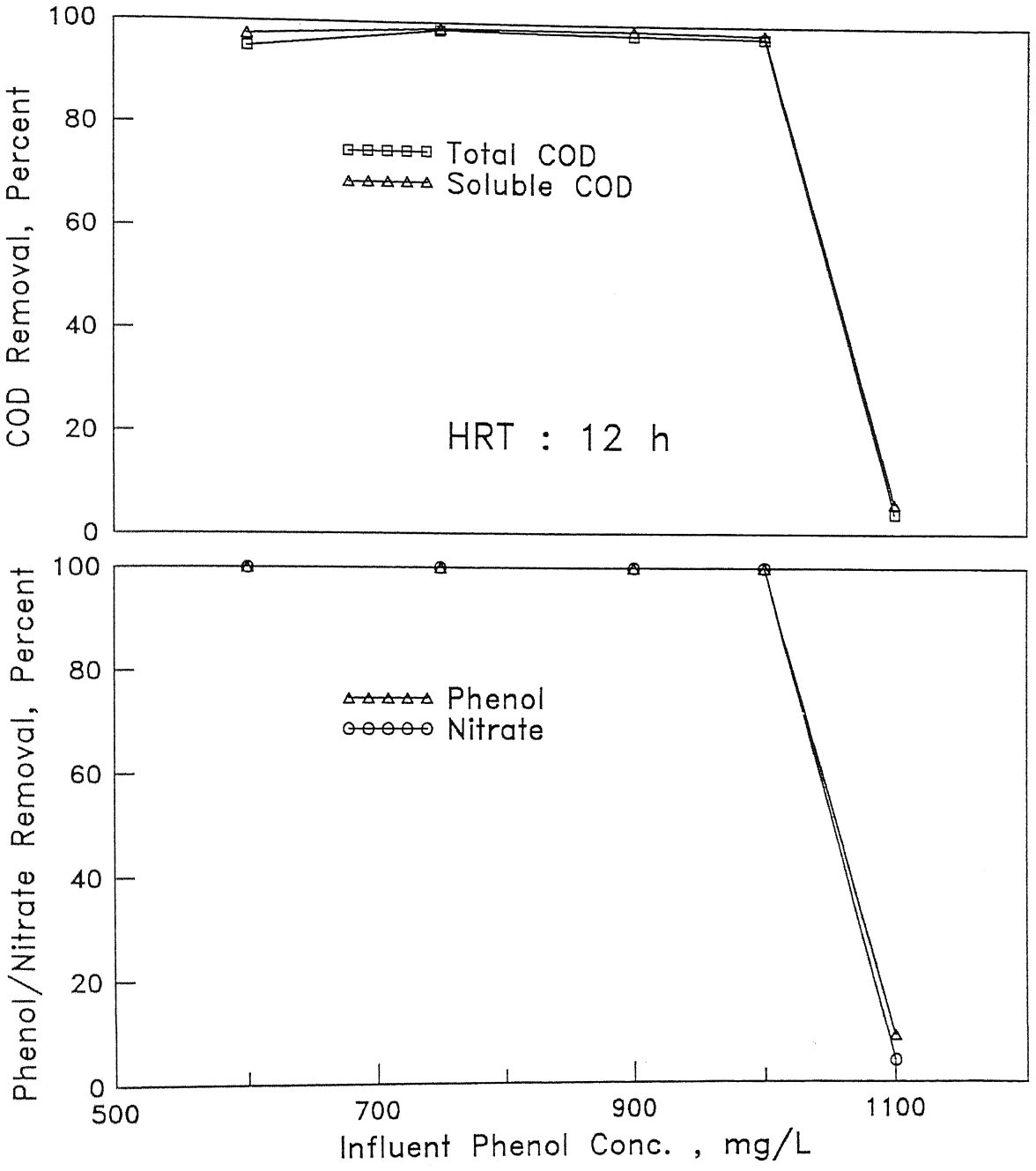


Fig. 5.8. Phenol, Nitrate, COD Removals as a function of Phenol Concentration

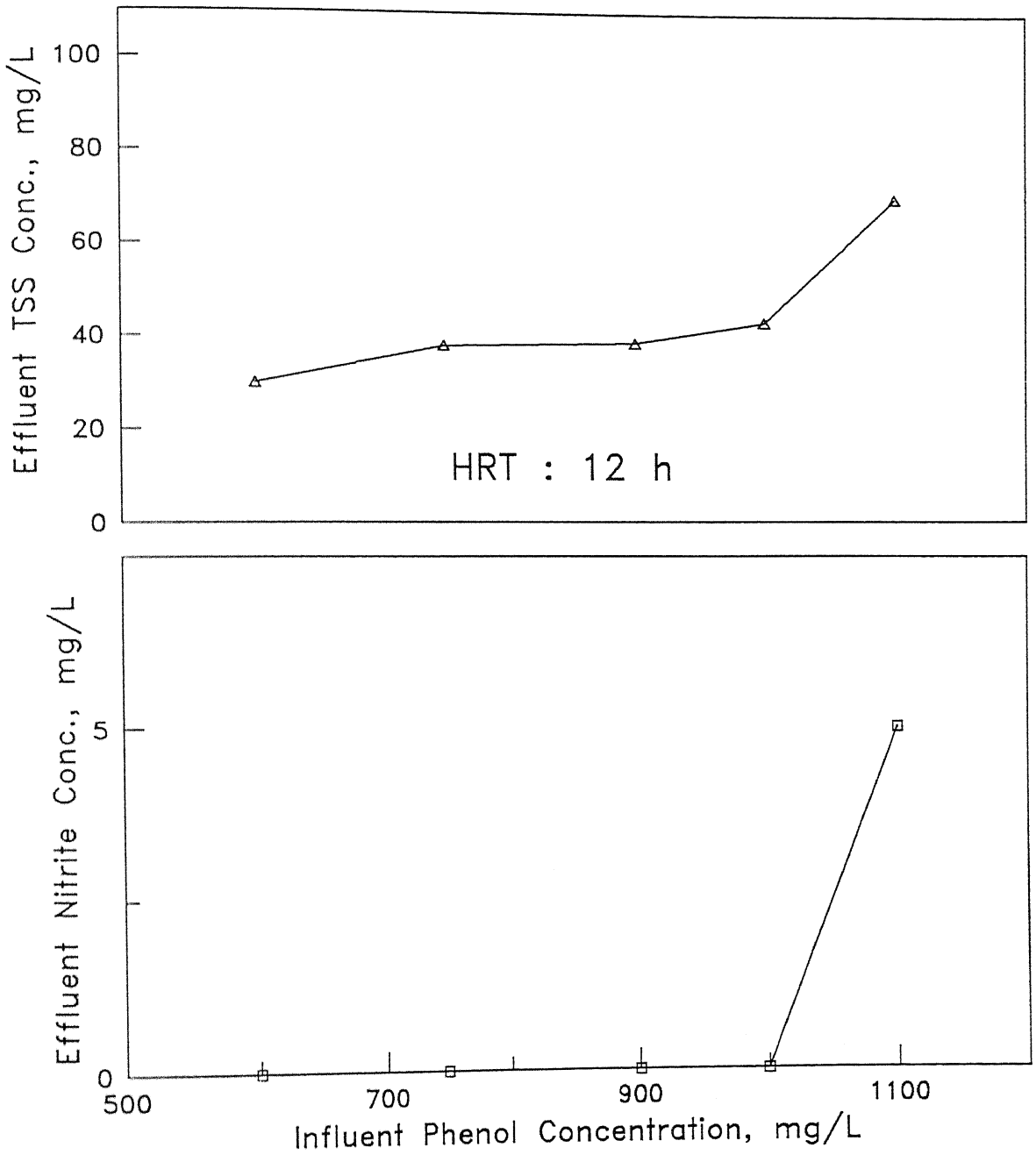


Fig. 5.9. Effluent Nitrite & TSS Conc.'s as a function of Phenol Conc.

Treatment efficiency of above 99 % could be achieved upto an influent phenol concentration of 1000 mg/L, indicating high process efficiency. However at 1100 mg/l, eventhough S.L.R. was only 5 g COD/L-d, phenol removal had decreased to 20 % .

This reduction in phenol removal could be due to inhibition of microbes by phenol toxicity. Pearson et al. (1980) reported similar inhibition in the activity of anaerobic phenol degrading microbes at phenol concentration of 1000 mg/L.

5.5 Investigations on Biomass Phase of the Reactor

Investigations on biomass were carried out to find out variations in biomass concentration along height of the rector. sludge samples from different sampling ports were drawn and analyzed for TSS and VSS. The micrographic examination of granular biomass was also carried out.

5.5.1 TSS and VSS Profile

TSS profile is illustrated in Figure 5.10. This shows the decrease of total suspended solids along the height of the reactor. The VSS profile is also shown in Figure 5.10. The volatile suspended solids were high in the lower part of the sludge blanket representing the biomass. The samples at different height depicted a VSS to TSS ratio of 0.93. This ratio is quite high compared to aerobic and anaerobic processes.

5.5.2 Micrographic Examination of Granular Biomass

Microstructure of the granules was examined on scanning electron microscope (SEM). The micrographs at different magnifications are presented in Figure 5.11. The first micrograph is showing the surface of one phenolytic denitrifying granule.

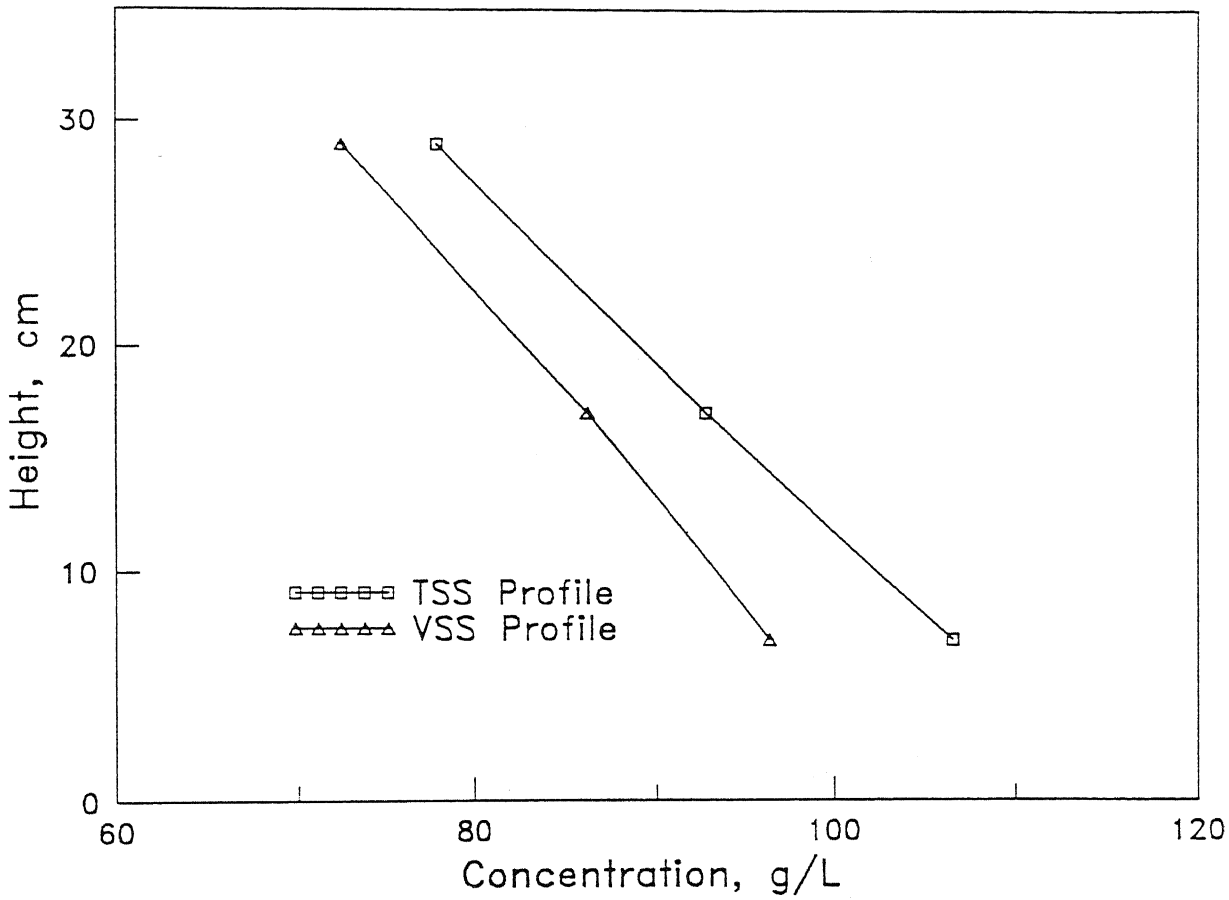
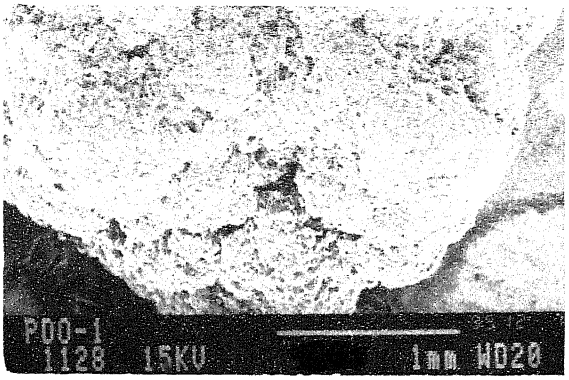
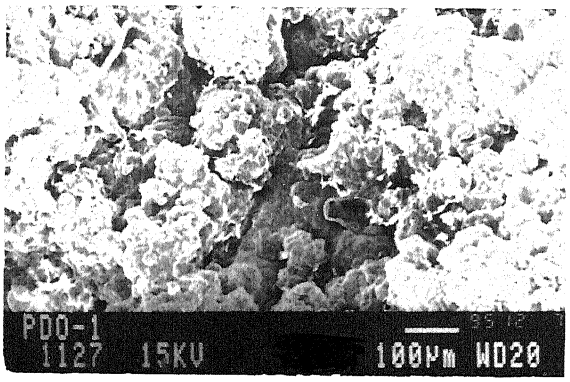


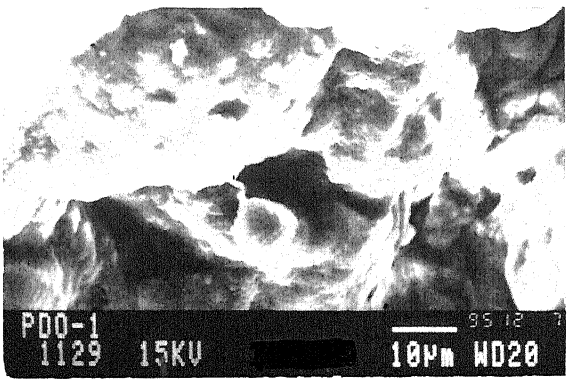
Fig. 5.10. Sludge Profiles



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ii



iii

Fig. 5.11 Scanning Electron Micrographs of Granular Phenolytic Denitrifiers at Different Magnifications

It gives a rough appearence. To get inside details two more photographs were taken at higher magnifications. The sturcture seem to be studded with bacterial microcolonies and arranged in clusters.

5.6 Investigations on Gaseous Phase

The gas samples were collected in the syringe and were analyzed using gas chromatograph with thermal conductivity detector using helium as carrier gas. The gas chromatographic analyses indicated 89-93 % N_2 and 7 to 11 % CO_2 in the gaseous products of the reactor. In the case of low loading rates of phenol the reaction occurring during anoxic denitrification may be presented as

$$5\ C_6H_5OH + 28\ NO_3^- \longrightarrow 14\ N_2 + 30\ CO_2 + H_2O + 28\ OH^-$$

The presence of N_2 in gaseous phase was due to conversion of NO_3-N to N_2 by phenolytic denitrifiers. A part of organic carbon of phenol is converted to CO_2 and some part is utilised for synthesis of biomass. The N_2 concentration in gas was quite high but CO_2 was low due to high dissolving tendency of CO_2 in water and thus escaping in the effluent.

6. CONCLUSIONS

The following conclusions may be drawn based on the results of present investigation.

1. Phenolytic granular sludge could easily be developed and maintained for a long time, without loss in its activity, at ambient temperatures on the minimal medium containing phenol as sole organic carbon source.
2. The UASB reactor with phenolytic granular biomass performed satisfactorily upto a space loading rate of 13 g COD/L-d at an influent phenol concentration of 750 mg/L. The failure occurred when space loading rate was increased to 16 g COD/L-d.
3. The phenolytic biomass can withstand a sludge loading rate of 0.26 g COD/g VSS-d with the resultant removal efficiency more than 80 %. Further increase in SLR to 0.30 g COD/g VSS-d resulted in the system failure.
4. Phenol concentration beyond 1000 mg/L is toxic to the phenol degrading organisms as indicated by system failure even at lower sludge loading rate.
5. Presence of nitrite in the effluent is the indication of stressed conditions and increase in its concentration reflects the deterioration of the system.
6. For influent phenol concentrations of 600 and 750 mg/L, the removal efficiency of 80 % or more could be achieved for HRT equal to or above 3 h.
7. The main gaseous products of the phenolytic denitrification are nitrogen (89-93 %) and carbon dioxide (7-11 %).

7. SUGGESTIONS FOR FUTURE WORK

The following suggestions are proposed for the future study.

1. Investigations may be undertaken with binary or tertiary mixtures of phenols crisol, catechol, resorcinol, pyragallol etc. as an organic carbon source for phenolytic denitrifiers as generally these are present in the industrial wastewaters.
2. A further study can be done to evaluate performance of UASB reactor at low phenol concentrations as these are emanated in various industries like oil refineries, petrochemicals etc.
3. The effect of varying C/N ratios in the feed on reactor performance may be studied as the present study was conducted with only one C/N ratio of 1.15.
4. A post-treatment to UASB may be incorporated as the present system can not conform to the maximum permissible concentration of phenols of 5 mg/L.

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